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Protective effects of *Cuminum cyminum* L. essential oil on ethylene glycol induced nephrolithiasis in mice

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ABSTRACT

Objective: To investigate the protective effect of *Cuminum cyminum* (*C. cyminum*) essential oil on ethylene glycol induced nephrolithiasis in mice.
Methods: The study comprised of the following four different groups of six mice: ethylene glycol group, *C. cyminum* group, treatment group and normal group. The levels of blood urea nitrogen and creatinine were analyzed and the kidney samples from all the animals of each group were stained with haematoxylin and eosin.
Results: Treatment group revealed mild tubular degeneration without formation of calcium oxalate crystals and protein deposition. There were no significant differences between serum levels of blood urea nitrogen and creatinine in treatment and normal groups.
Conclusions: It seems that *C. cyminum* essential oil significantly decreased formation of calcium oxalate crystals and the growth of renal calculi in different parts of the tubules.

1. Introduction

Urinary calculi are the third prevalent disorder in the urinary system and may cause obstruction, hydronephrosis, infection and hemorrhage in the urinary system. Most calculus in the urinary system arise from a common component of urine, *e.g.* calcium oxalate (CaOx), representing up to 80% of analyzed stones[1,2]. Kidney stone formation consists of several stages including supersaturation, nucleation, growth, aggregation and retention within renal tubules[3]. Surgical operation, lithotripsy, and local calculus disruption using high-power laser are widely used to remove the calculi. However, these procedures which are highly cost-effective may cause severe complications[4]. Herbal medicines have several phytoconstituents and exert their beneficial effects in urolithiasis by multiple mechanisms[5].

Cumin is a strong aromatic dried ripe fruit seed of *Cuminum cyminum* L. (*C. cyminum*). It belongs to the Apiaceae family (Parsley family). Cumin seeds are ancient spices with a strong aromatic smell and warm bitter taste. Cumin not only is a spice but also has great medicinal value. Cumin is widely used in traditional medicine to treat flatulence, digestive disorders, diarrhea and wounds. It is valuable in dyspepsia, diarrhea and hoarseness, and used as a remedy against indigestion and colic[6]. In recent years, herbal medical researches have focused on therapeutic effects of *C. cyminum*. The researches showed that fruit of the plant have anticonvulsant and analgesic activity[7-9]. There are some reports regarding the antibacterial, anti-diabetic and estrogenic activities of this plant[10-12].

The present study was conducted to investigate the protective effect of *C. cyminum* essential oil on ethylene glycol induced nephrolithiasis in mice.

2. Material and methods

2.1. Animals

The experimental protocols were approved by the Research Ethic Committee of the Shahid Bahonar University of Kerman, Iran. A total of 24 male NMRI mice were purchased from the Animal Laboratory of Kerman University of Medical Sciences, Kerman, Iran and kept in the Center for Laboratory Animal Care at the Veterinary Medicine Faculty of Shahid Bahonar University of Kerman for 1 week before treatment. The mice weighed 25–30 g and were at the same age of 1.5–2 months old. The experimental animals were randomly divided into four groups of 12 animals and were housed in standard polypropylene cages with wire mesh top, at 21 °C in a 12 h/12 h dark-light cycle. During the study, the animals

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All experimental procedures were considered carefully in accordance to KUMS guide for the use and care of laboratory animals and approved by the Ethics Committee of KUMS.

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received water and pellet food (Javaneh Khorasan Co, Iran) *ad libitum*.

2.2. Experimental design

The study comprised of following four different groups of six mice: ethylene glycol group was received 0.1 mL normal saline by gavage and 1% ethylene glycol (Merck, Darmstadt, Germany) in drinking water for 35 days, *C. cyminum* group was received 0.1 mL *C. cyminum* essential oil (Barij Essence, Kashan, Iran) at a dose of 1 mg/kg by gavage and normal saline as drinking water for 5 weeks, treatment group was received 1% ethylene glycol (Merck, Darmstadt, Germany) in drinking water and treated by *C. cyminum* essential oil (1 mg/kg) during experimental period and normal group was received the same volume of normal saline during mentioned period.

2.3. Biochemical assays

The blood samples were collected directly from the animals by heart puncturing. The samples were centrifuged at 10000 r/min for 15 min and the clear serum were collected and stored in a -20 ˚C freezer. The levels of blood urea nitrogen (BUN) and creatinine were analyzed. All biochemical assays were determined according to Pars Azmon kits (Pars Azmon Co, Iran) by Autolab auto-analyzer (Rome, Italy).

2.4. Histopathological assays

After necropsy, the kidney samples from all the animals of each group were preserved in 10% neutral buffered formalin solution for histological examination at the end of the experiment. Formalin-fixed samples were processed by the standard paraffin wax technique, and sections of 5 cm thickness were cut and stained with haematoxylin and eosin (H&E).

2.5. Statistical analysis

All data were expressed as mean ± SE. Statistical analysis was performed using One-way ANOVA, followed by *post-hoc* Tukey HSD test. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Biochemical assay

Results of serum biochemical parameters were presented in Table 1. The data obtained revealed that serum levels of BUN and creatinine in ethylene glycol group were significantly increased ($P < 0.05$) in comparison with normal group and there were no significant differences between treatment and normal groups.

Table 1

Comparison of biochemical parameters in all groups[a] (mg/dL).

Parameters	Normal group	Group Cc	Group E	Treatment groups
BUN	56.70 ± 3.10	61.80 ± 1.70	211.50 ± 5.70[a]	68.60 ± 4.20
Creatinine	0.76 ± 0.12	0.74 ± 0.20	2.34 ± 0.41[a]	0.71 ± 0.09

[a]: A significant difference in comparison with normal group ($P < 0.05$); Group Cc: *C. cyminum* group; Group E: Ethylene glycol group.

3.2. Histopathological examination

Histopathological examination of kidney revealed the normal glomeruli and tubules with the absence of CaOx crystals in the normal group (Figure 1). In ethylene glycol group, some renal corpuscles had atrophied glomerular tufts and dilated urinary space. The epithelium of renal tubules showed degenerative changes including cell swelling and vacuolar degeneration (Figure 2). Excreted proteins were mostly deposited in the lumen of tubules in the cortex. These depositions were eosinophilic or basophilic due to mixed with calcium compounds (Figure 3). Depositions of CaOx crystals were observed in some tubules (Figure 4). Treatment group revealed mild tubular degeneration without formation of CaOx crystals and protein deposition (Figure 5).

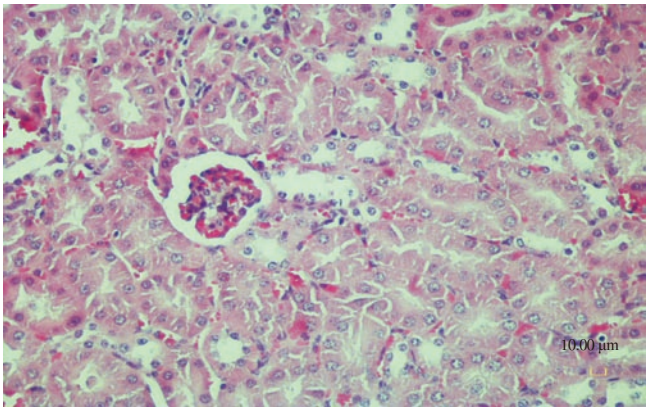

Figure 1. Histology of kidney showing the normal glomeruli and tubules in normal group (H&E, bar = 10 µm).

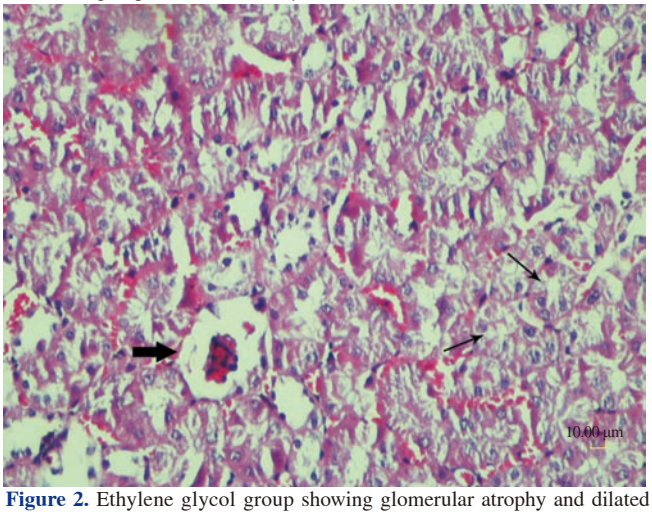

Figure 2. Ethylene glycol group showing glomerular atrophy and dilated urinary space (thick arrow) as well as vacuolar degeneration of renal tubules (thin arrows) (H&E, bar = 10 µm).

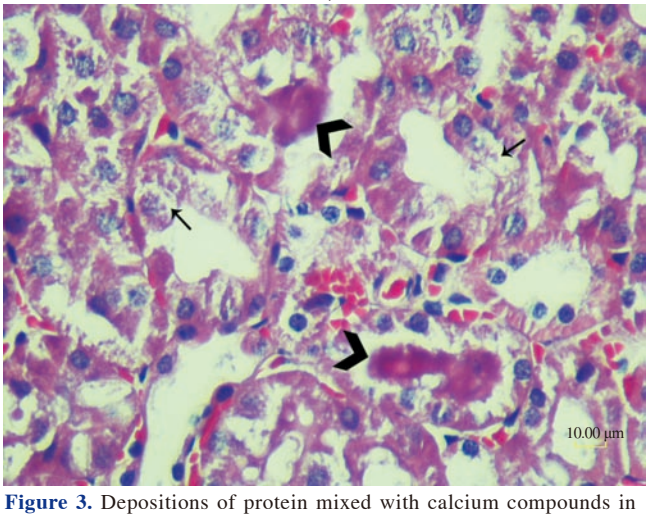

Figure 3. Depositions of protein mixed with calcium compounds in tubules (arrow heads) and vacuolar degenerations of tubules (arrows) of ethylene glycol group (H&E, bar = 10 µm).

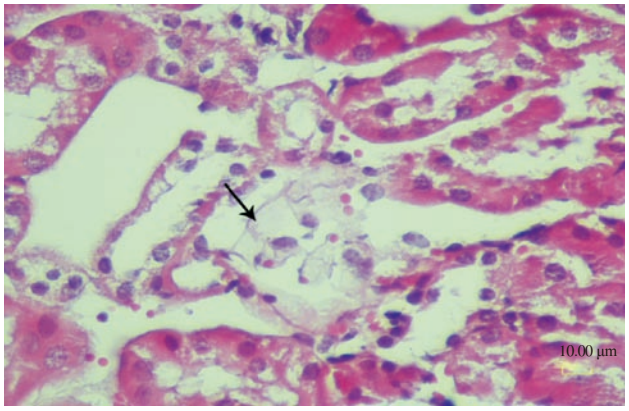

Figure 4. Visible CaOx crystals in the lumen of the renal tubule (arrow) in ethylene glycol group (H&E, bar = 10 µm).

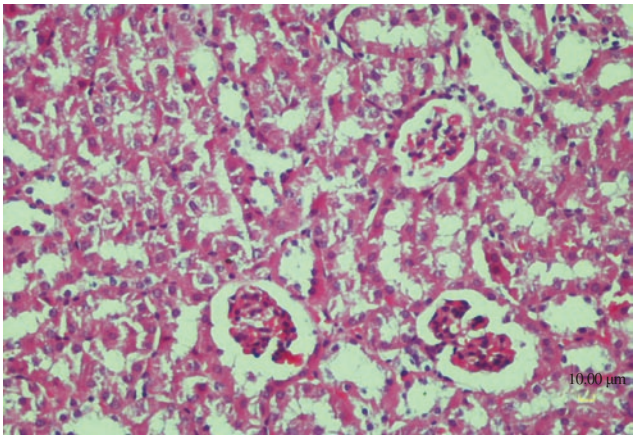

Figure 5. Treatment group only showing mild tubular degeneration (H&E, bar = 10 µm).

4. Discussion

According to the Khan and Thamilselvan, CaOx crystals can produce damages in the epithelial cells of nephrons, and consequently, the cells may produce some products which cause the aggregation of crystals[13].

Some of the constituents of the essential oil of *C. cyminum* such as α-pinene and β-pinene have been reported to possess anti-inflammatory activity. Another constituent, myrcene, has peripheral analgesic effect acting by the stimulation of nitric oxide pathway[9]. Also the seeds of *C. cyminum* have a high antioxidant activity[6].

El-Dakhakhny *et al*., stated that anti-inflammatory and antioxidant effects of the different compounds of the essential oil of *C. cyminum* play an important role in the treatment of CaOx calculi and related nephritis[14]. It seems that CaOx crystals can damage epithelial cells and induce inflammation[14].

The exposure of CaOx crystals induces the production of some inflammatory molecules with no apparent role in crystal formation. These indicate a relationship between nephrolithiasis and inflammation[15].

Kramer *et al*. reported that CaOx calculi may have a bacterial origin[16]. Antibacterial activity of the essential oil of *C. cyminum* may be effective in treatment of calculi formation[10].

Obstruction of the outflow of urine due to calculi formation decreases the glomerular filtration rate and the accumulation of waste products in blood[17]. In the present research, the nephrotoxicity induced by ethylene glycol was characterized by elevation of BUN and serum creatinine. But above mentioned parameters were decreased in the treatment group compared to the normal group. It seems that mentioned repair is due to protective effects of the essential oil of *C. cyminum*.

It seems that *C. cyminum* essential oil significantly decreased the formation of CaOx crystals, and the growth of renal calculi in different parts of the tubules. The results confirm folk information regarding antiurolithiatic activity of the medicinal plants.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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References

[1] Türk C, Knoll T, Petrik A, Sarica K, Skolarikos A, Straub M, et al. Guidelines on urolithiasis. Arnhem: European Association of Urology; 2015. [Online] Available from: http://uroweb.org/wp-content/uploads/22-Urolithiasis_LR_full.pdf [Accessed on 4th August, 2015]

[2] Yiu AJ, Callaghan D, Sultana R, Bandyopadhyay BC. Vascular calcification and stone disease: a new look towards the mechanism. *J Cardiovasc Dev Dis* 2015; **2**: 141-64.

[3] Aggarwal KP, Narula S, Kakkar M, Tandon C. Nephrolithiasis: molecular mechanism of renal stone formation and the critical role played by modulators. *Biomed Res Int* 2013; **2013**: 292953.

[4] Coe FL, Evan A, Worcester E. Kidney stone disease. *J Clin Invest* 2005; **115**: 2598-608.

[5] Yadav RD, Jain SK, Alok S, Mahor A, Bharti JP, Jaiswal M. Herbal plants used in the treatment of urolithiasis: a review. *Int J Pharm Sci Res* 2011; **2**(6): 1412-20.

[6] El-Ghorab AH, Nauman M, Anjum FM, Hussain S, Nadeem M. A comparative study on chemical composition and antioxidant activity of ginger (*Zingiber officinale*) and cumin (*Cuminum cyminum*). *J Agric Food Chem* 2010; **58**: 8231-7.

[7] Sayyah M, Peirovi A, Kamalinejad M. Anti-nociceptive effect of the fruit essential oil of *Cuminum cyminum* L. in rat. *Iran Biomed J* 2002; **6**(4): 141-5.

[8] Tremont-Lukats IW, Megeff C, Backonja MM. Anticonvulsants for neuropathic pain syndromes: mechanisms of action and place in therapy. *Drugs* 2000; **60**: 1029-52.

[9] Bhat SP, Rizvi W, Kumar A. Effect of *Cuminum cyminum* L. seed extracts on pain and inflammation. *J Nat Remedies* 2014; doi: 10.18311/jnr/2014/81.

[10] Jazani NH, Zartoshti M, Shahabi S. Antibacterial effect of Iranian *Cuminum cyminum* essential oil on burn isolates of *Pseudomonas aeruginosa*. *Int J Pharmacol* 2008; **4**: 157-9.

[11] Srivsatava R, Srivastava SP, Jaiswal N, Mishra A, Maurya R, Srivastava AK. Antidiabetic and antidyslipidemic activities of *Cuminum cyminum* L. in validated animal models. *Med Chem Res* 2011; **20**(9): 1656-66.

[12] Patil SR, Patil MB. Evaluation of estrogenic activity of various extracts of *Cuminum cyminum* seeds in female rats. *J Cell Tissue Res* 2012; **12**(1): 3055-60.

[13] Khan SR, Thamilselvan S. Nephrolithiasis: a consequence of renal epithelial cell exposure to oxalate and calcium oxalate crystals. *Mol Urol* 2000; **4**: 305-12.

[14] El-Dakhakhny M, Madi NJ, Lembert N, Ammon HP. *Nigella sativa* oil, nigellone and derived thymoquinone inhibit synthesis of 5-lipoxygenase products in polymorphonuclear leukocytes from rats. *J Ethnopharmacol* 2002; **81**: 161-4.

[15] Khan SR, Kok DJ. Modulators of urinary stone formation. *Front Biosci* 2004; **9**: 1450-82.

[16] Kramer G, Klingler HC, Steiner GE. Role of bacteria in the development of kidney stones. *Curr Opin Urol* 2000; **10**: 35-8.

[17] Adeneye AA, Benebo AS. Protective effect of the aqueous leaf and seed extract of *Phyllanthus amarus* on gentamicin and acetaminophen-induced nephrotoxic rats. *J Ethnopharmacol* 2008; **118**: 318-23.